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SODIUM DEPENDENCE OF THE INWARD SPREAD OF ACTIVATION IN ISOLATED TWITCH MUSCLE FIBRES OF THE FROG

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SUMMARY

1. The excitatory process travelling along the T-system may be either electrotonic or regenerative. If Na^+ dependent action potential is present in the tubular membranes, high frequency of stimulation might cause a Na^+ depletion in the tubules sufficient to abolish this process.

2. We tested this hypothesis by recording tension in isolated muscle fibres stimulated tetanically (up to 60 shocks/sec). In low $[Na^+]$ solutions, output tension was initially similar to that in normal Ringer, but then fell smoothly to a substantially lower value.

3. The activity of individual myofibrils was recorded directly with cinémicrographs during isotonic contractions while the fibres were stimulated at high frequencies. In low $[Na^+]_o$ wavy myofibrils appeared in the centre of the fibre and spread towards the periphery, indicating failure of activation. Wavy myofibrils never appeared in normal Ringer.

4. Intracellular action potentials recorded during the tetanic stimulation indicated that the inactivated myofibrils present in low $[Na^+]$ solutions cannot be explained by the changes in size and duration of the action potential.

5. Our results strongly suggest the existence of a regenerative Na^+ conductance in the tubular membrane during the inward spread of an excitatory process.

INTRODUCTION

There is little doubt that the transverse tubular system constitutes the means of carrying electrical information from the surface towards the

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centre of the striated muscle fibre (Huxley & Taylor, 1958; Gage & Eisenberg, 1969). The mechanism by which the electrical signal is conducted to the centre of the fibre is not yet firmly established. In general there are two possibilities. The first is that an action potential propagates down the tubules, and the second is that the action potential is confined to the surface membrane, and spreads along the tubules in a purely electrotonic fashion. Falk & Fatt (1964), Falk (1968), Adrian, Chandler & Hodgkin (1969) have proposed some models for electrotonic spread along the tubular network. Based on the observed radial spread of contraction with controlled surface depolarization, Adrian, Costantin & Peachey (1969) proposed that at room temperature the electrical characteristics of the T-system are such that a full size action potential is just sufficient to activate by electrotonic spread the central myofibrils. Gonzalez-Serratos (1966, 1967, 1968, 1971) measured the velocity of the inward spread of activation and its temperature dependence. He suggested that his results are more compatible with the idea of a regenerative process rather than a passive one along the tubules. Recently Costantin (1970) and Costantin & Taylor (1971) have shown that the mode of the radial spread of the activation is affected by the presence of tetrodotoxin and dependent on the external sodium concentration. Costantin pointed out that these results are consistent with the idea of a sodium conductance change at the level of the tubular membrane which contributes to the inward spread of the electrical signal along the T-system. Thus, the evidence at this stage is that electrotonic spread is sufficient for full activation under normal conditions, but it seems a very good possibility that the tubules, nonetheless, have an action potential mechanism.

The results of Adrian, Costantin & Peachey (1969) lead to an interesting prediction. If spread along the tubules is purely passive, then decreasing action potential amplitude by lowering the external Na^+ concentration ($[Na^+]_o$) should lead to incomplete activation of the fibre, and thus should diminish twitch tension. On the other hand, if there is an action potential in the tubules, twitch tension should be unchanged when $[Na^+]_o$ is lowered, as long as there is sufficient Na^+ for an action potential, but by fast repetitive stimulation it might be possible to deplete the tubules of Na^+. This could be detected by a progressive drop of tension during a tetanus. These considerations prompted us to analyse the effects of low $[Na^+]_o$ on the contractile performance of isolated muscle fibres stimulated at various frequencies. Although we have not developed a precise quantitative model for our results, they certainly suggest that tubular Na^+ depletion does occur and that tubular membrane undergoes an increase in Na^+ permeability during activation, a process which may be regenerative in nature.

METHODS

Some experiments were performed on single fibres dissected from the semitendinosus muscle and others on whole sartorius muscle from *Rana pipiens*.

Isometric tension measurements

After dissection, the fibres were mounted in a chamber similar to the one described by Lüttgau (1965). One tendon was gripped by a stainless steel clamp and the other was hooked to a lever connected to a force transducer (RCA 5734) for recording isometric tension. Two electrodes for external recording were connected to a cathode follower. The output of the cathode follower was displayed together with the output of the tension transducer on a dual beam oscilloscope. Photographic records were obtained at low sweeping speed with a Polaroid camera. At the same time another oscilloscope was used to record the events in a moving film at 10 cm/sec.

Isotonic recording system

When ciné recordings were taken, the isolated muscle fibre was mounted in the middle of a 3 mm wide and 3 mm deep channel of a cell made of methacrylate polymer (Perspex). The pelvic tendon was clamped near one end of the trough and two platinum wires used as cathodes were set across the bottom of the channel near this end. The distance between the electrodes was around 3 mm. Two other platinum wires located at each end of the channel were used as anodes. The tibial tendon which was several millimetres long was fixed to a moving lever of the type described elsewhere (Gonzalez-Serratos, 1965). This allowed the muscle to undergo isotonic contraction. The chamber was fixed on the stage of an ordinary light microscope. The ciné-micrographs were taken at 100 frames/sec with either a × 25 water immersion objective, N.A. 0·40 or a × 40 water immersion objective, N.A. 0·75 (Zeiss) and a × 6 eyepiece. The recordings were done from a point of the fibre located between the two cathodes and at 1–3 mm from the pelvic tendon. Further details of the ciné recordings have been described previously (Gonzalez-Serratos, 1971).

Intracellular recording

In some experiments action potentials were recorded intracellularly during tetanic stimulation. By means of two fine external electrodes, we could stimulate only one or two fibres on the surface of a sartorius muscle. Under these conditions we could obtain a continuous record of membrane potential from one of the stimulated fibres without damaging it by using the elegant floating recording electrodes described by Colomo & Rocchi (1965).

The micropipette was connected through an Ag–AgCl electrode to the input of a cathode follower with negative-capacitance compensation (W. P. Instruments). In order to see the time course of the changes of the action potential during stimulation low speed recordings were photographed with a Polaroid camera. The individual action potentials were analysed from records photographed in a moving film camera (Grass Instruments Co.) at 50 cm/sec. In this series of experiments when after repetitive stimulation there was not good recovery of the resting and action potentials, the run was discarded.

Solutions

The normal Ringer solution had the following composition (Adrian, 1956), m.mole/l. NaCl 115; KCl 2·5; $CaCl_2$ 1·8; NaH_2PO_4 0·85; Na_2HPO_4 2·15. Low sodium solutions

were prepared by replacing NaCl by Tris chloride or LiCl in equimolar amounts or by sucrose on a 1:1·57 molar basis (Gage & Quastel, 1966). Fibres were kept in a given solution for at least 10 min before any experimental procedure was started.

RESULTS

Isometric tension measurements

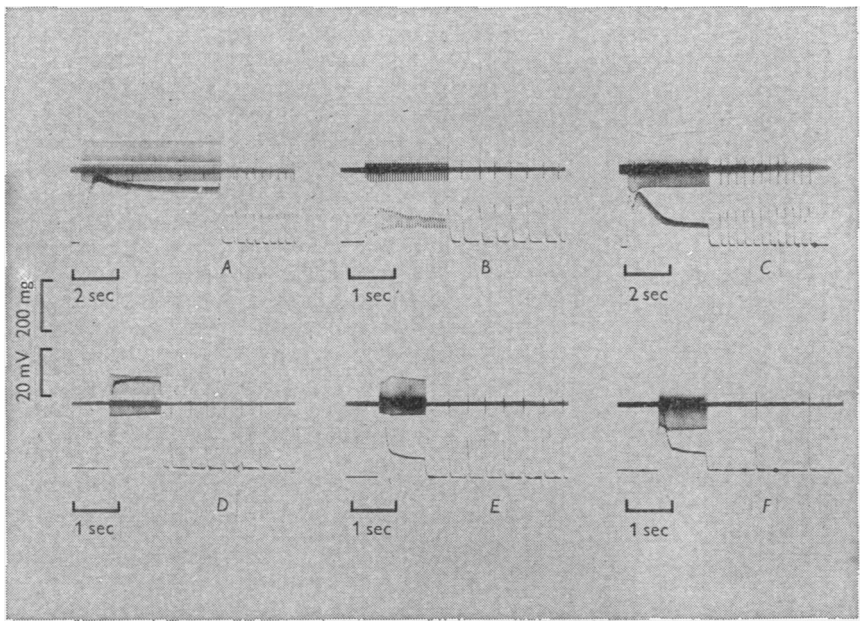
Text-fig. 1 shows the results obtained by stimulating a single fibre at 18 shocks/sec or 60 shocks/sec in media of different composition. The only purpose of the external currents recordings was to ensure that the fibre responded electrically to each stimulus. The shape and size of these action currents are difficult to interpret and physiologically they do not mean much in the experiments reported on here. In panel *A* and *D* the fibre was bathed in normal Ringer solution. It can be observed that at 18 shocks/sec (*A*) tetanic fusion was not reached, and for this particular fibre the maximal tension dropped about 15 % after reaching its peak value. When stimulated at 60 shocks/sec (panel *D*) the peak tetanic tension was well maintained throughout the stimulation period.

Panels *B* and *E* show the results obtained when the fibre was stimulated at the same frequencies while bathed in a medium containing 62·5 mM-$[Na^+]_o$ with the remainder of the Na^+ substituted by Tris. It is clear that in both cases the peak tension drops rapidly toward a steady-state value lower than the one obtained in normal Ringer solution. In panel *B* it is observed that the maximum value of the peak tension in 62·5 mM-$[Na^+]_o$ was not as big as in the normal Ringer solution. In panel *E*, however, the maximum tension value was initially about the same as the one obtained in normal Ringer solution at the same frequency but it fell to a 22·5 % with a half-time of 0·14 sec. This drop in tetanic tension was not due to an irreversible change in the fibre, for invariably it did not appear on return to normal Ringer. Panels *C* and *F* show a similar run in a solution in which 62·5 mM-NaCl were substituted by an isosmotic amount of sucrose. The results are qualitatively similar to those obtained with part of the Na^+ replaced by Tris. This shows that the fall in tension is not due to a specific effect of the Tris containing solution.

It is well known that Li^+ can effectively replace Na^+ in the generation and propagation of the action potential (Keynes & Swan, 1959). Text-fig. 2 shows that substituting 77 mM-Na^+ by Li^+ does not adversely affect the tetanic tension during the stimulation period.

The records shown in Text-fig. 1 suggest that the rate at which the tension declines during repetitive stimulation increases with the frequency. We examined this more carefully, and Text-fig. 3 shows the results we obtained during an experiment in which a single fibre was stimulated at

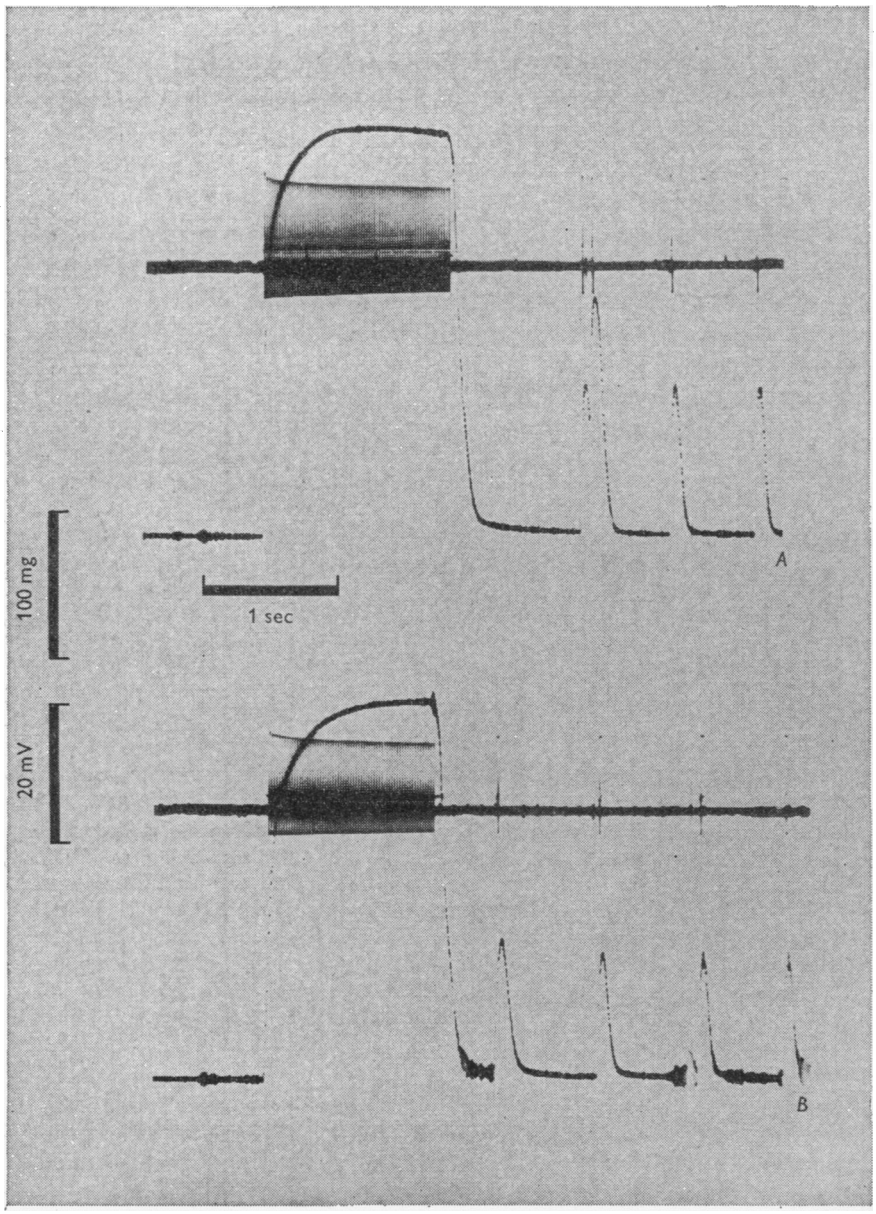
different frequencies (60, 20 and 12 shocks/sec) while bathed in different $[Na^+]_o$ (120, 62·5 and 43 mM). For each Na^+ concentration the peak tension increases with the stimulation frequency. In normal Ringer solution, as shown before, there is no decline in tension during the stimulation period. On the contrary, at lower stimulation frequency a biphasic increase in



Text-fig. 1. Development of tension (lower traces) of an isolated muscle fibre stimulated at 18 shocks/sec (*A–C*) and at 60 shocks/sec (*D–F*) in normal Ringer solution (*A* and *D*) and in a solution containing 62·5 mM-Na^+ (*B*, *C*, *E* and *F*). In *B* and *E* the NaCl was substituted by Tris Cl and in *C* and *F* by sucrose so as to keep them isosmotic with respect to the normal Ringer. The upper trace in each panel shows the action currents recorded during the stimulation. After each tetanic stimulation single twitches are shown which indicate that the decline in tension observed in the low $[Na^+]_o$ solutions were not due to damage of the fibre. Fibre 27. iv. 71 (*a*).

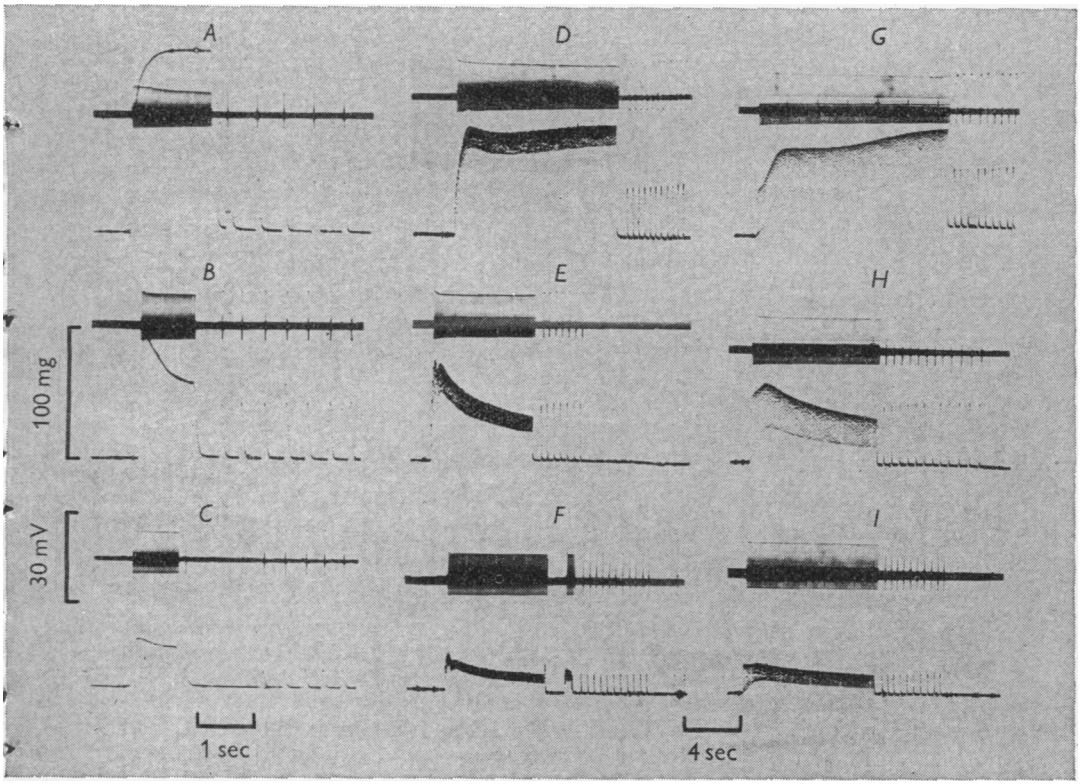
tension is observed. In 62·5 mM and 43 mM-$[Na^+]_o$ a decline of tension during the stimulation period is present at all the frequencies studied and the rate of tension fall appears to increase monotonically with the stimulation frequency.

In this type of experiments the steady level of tension was nearly reached at the end of the high frequency stimulation periods in low $[Na^+]_o$ solutions. The final plateau tension was estimated by slightly extrapolating the tension decay.



Text-fig. 2. Upper and lower traces as in Text-fig. 1. In *A* the bathing solution contained 43 mM-Na^+ and 77 mM-Li^+. In *B* the fibre was in normal Ringer solution. In both cases the fibre was stimulated at 60 shocks/sec. Fibre: 27. iv. 71 (*b*).

In doing so, the half-time for the decay between the peak tension and the estimated plateau tension can be measured. In this particular experiment, the values obtained in 62·5 mM-$[Na^+]_o$ were 0·26, 1·89 and 2·74 sec for 60, 20, and 12 shocks/sec respectively. In 43 mM-$[Na^+]_o$ the peak ten-



Text-fig. 3. Upper and lower traces as in Text-fig. 1. The frequencies of stimulation and the Na concentrations in the bathing media were as follows:

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
mM $[Na^+]_o$	115	62	43	115	62	43	115	62	43
shocks/sec	60	60	60	20	20	20	12	12	12

In the solutions containing less than 115 mM-$[Na^+]_o$, NaCl was substituted m-mole for m-mole with Tris Cl. Some action currents seem to fail. It can be noticed that this is an artifact due to the coincidence of the action current recorded and the oscilloscope graticule. Fibre: 25. iv. 71.

sion is considerably smaller than in the higher $[Na^+]_o$ media, and this makes it more difficult to observe the decay of the tension during the stimulation, since the initial and final values of tension are not greatly different. However, it is possible to appreciate the effect of the stimulation

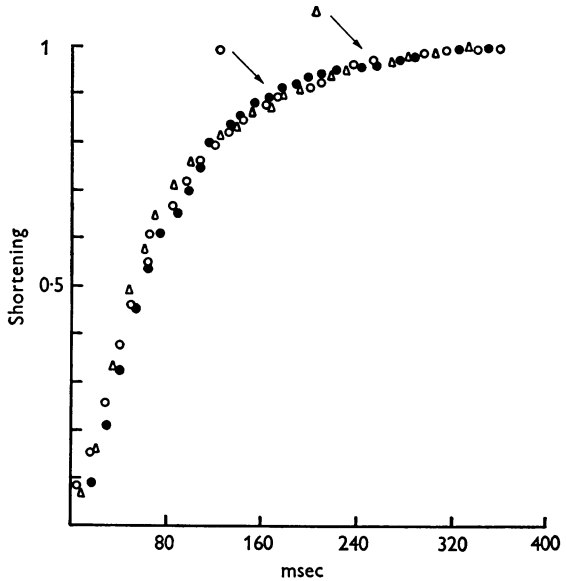
frequency. The half time of decay are 0·23, 2·0 and 3·26 sec for 60, 20 and 12 shocks/sec respectively. This experiment also shows that the final level toward which the tension tends during repetitive stimulation is less the lower the $[Na^+]_o$ and the higher the frequency of stimulation.

The activation of the myofibrils under isotonic condition

To see if the drop in tension was due to failure of activation of some of the myofibrils, we took ciné-micrographs of fibres that were shortening isotonically. Under these conditions inactive myofibrils became wavy as they are passively compressed by surrounding active myofibrils. This was done by repeating the experiments with fibres under similar conditions as before, but now shortening was allowed and ciné-micrographs were taken at the same time. The rationale behind these experiments is that if the drop in tension is due to the failure of activation of some of the myofibrils, these should relax and become wavy provided that the rest of the fibre remained shortened as described by Huxley & Gordon (1962) and Gonzalez-Serratos (1965). In normal Ringer solution there were only faint suggestions of wave formation rather evenly distributed throughout the optical section, even after 6 sec of stimulation at 70 shocks/sec. From the absence of visible sarcomere spacings in Pl. 1*B*, it can be inferred that sarcomere length was 1·5 μm or less. If, on the other hand, the fibre was stimulated in a solution containing 62·5 mM-$[Na^+]_o$, the centre (and only the centre) of the fibre became obviously wavy (Pl. 1*D* and Pl. 2). There are visible sarcomere spacings in the centre of the fibre in Pl. 1*D* and the apparent sarcomere spacing measured parallel to the waves is about 1·9 μm. This shows that lowering $[Na^+]_o$ had no adverse effect on the ability of the fibre to shorten. Further evidence that $[Na^+]_o$ did not affect shortening is given in Text-fig. 4. Shortening was measured by following the progress of a piece of connective tissue attached to the fibre. It is clear that lowering the $[Na^+]_o$ had no effect on either the rate or the extent of shortening. The symbols indicate the time when wave formation began in 62·5 and 51 mM-$[Na^+]_o$. The waves became steadily more prominent and spread towards the periphery as stimulation continued, even after shortening of the fibre had reached a maximum.

The time at which wave formation began in these isotonic experiments depended on both stimulus frequency and $[Na^+]_o$. For a fibre in 62·5 mM-$[Na^+]_o$ stimulated at 70 shocks/sec, the waves appeared about 200 msec after the beginning of stimulation. At 50 shocks/sec in the same solution, the waves appeared 260–290 msec after the beginning of stimulation (Pl. 2). At 50 shocks/sec it took only 160 msec for the waves to appear with a fibre in 51 mM-$[Na^+]_o$. In general, the beginning of waviness occurred at about the same time that tension began to drop in the isometric experiments.

This leads us to believe that the central myofibrils in the isotonic experiments were active at the beginning of stimulation, and became inactive sometime after, just as in the isometric experiments.



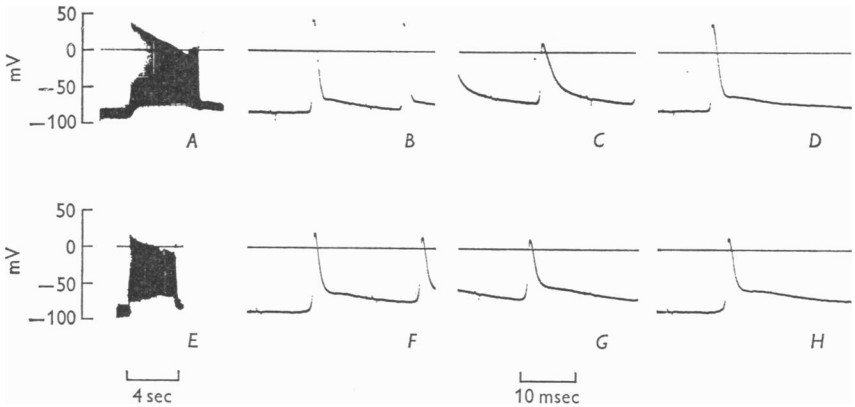
Text-fig. 4. Time course of shortening of an isolated muscle fibre stimulated at 50 shocks/sec in media of different Na^+ content. Filled circles, 115 mM-$[Na^+]_o$; triangles, 62·5 mM-$[Na^+]_o$; open circles, 51 mM-$[Na^+]_o$. The measurements were done by following the movement of pieces of connective tissue through the field of view in a point close to the fixed tendon. Although the amount of shortening was similar in each case in order to compare them the maximum shortening has been taken as one. Arrows indicate the moment when wavy myofibrils appear. Fibre 25. v. 71.

Intracellular recording of the action potential during repetitive stimulation

It is possible that the tension begins to drop during stimulation of fibres in low $[Na^+]_o$ simply because the action potential in the surface membrane becomes too small to activate the central myofibrils. To see whether this might be the case, we made intracellular recordings of the action potentials of tetanically stimulated fibres of sartorius muscles as described in Methods pp. 4–5. For a fibre in normal Ringer, action potential amplitude decreases as stimulation proceeds (Text-fig. 5*A*). After several seconds of stimulation the action potential is so diminished that it has no overshoot, and membrane potential at the peak is several millivolts negative. Toward the end of the stimulation period in Text-fig. 5*A*, the fibre began to respond only to alternate stimuli, and at this time action potential

amplitude began to increase. Duration of the action potential increased as it became smaller (Text-fig. 5*C*, as compared with Text-fig. 5*A*). These results are in agreement with the findings reported by Colomo & Rocchi (1965) and Hanson & Persson (1971).

In 62·5 mM-$[Na^+]_o$ the initial amplitude of the action potential is less than in normal Ringer, and amplitude then declines as stimulation (50 shocks/sec) proceeds. When extrapolated to the semitendinosus muscles



Text-fig. 5. Action potentials in normal Ringer solution (*A–D*) and in 62·5 mM-$[Na^+]_o$ solution (*E–H*) during stimulation at 60 and 50 shocks/sec respectively. In *A* and *E* the records were taken at very low speed to show the envelopes of changes in magnitude of the action potentials during the period of stimulation. In *G* an action potential occurring 290 msec after the beginning of the stimulation is illustrated; in *C*, 2·5 sec after the beginning of the stimulation, an action potential of similar magnitude is shown. *B* and *F* show the first two action potentials of the run. *D* and *H* illustrate action potentials several minutes after the run ended. The zero potential level is indicated by the horizontal lines. Fibre 29.x.71.

in which our tension recordings were made, these results indicate that changes in the action potential play no part in the dropping off of tension in low $[Na^+]_o$ solutions. Fibres in normal Ringer show no drop in tension, even when, judging from the sartorius recordings (Text-fig. 5*A*), their action potential do not reach 0 mV. In low $[Na^+]_o$ solutions, tension in semitendinosus fibres is clearly down and waves are beginning to appear in the ciné-micrographs 290 msec after the commencement of stimulation. The action potential at this time, again judging from the sartorius records, still has an overshoot (13 mV in the record of Text-fig. 5*G*). Thus action potential diminution alone is not sufficient to explain decreased tension, since a fibre in normal Ringer produces full tension even when its action potential is smaller than the one shown in Text-fig. 5*G*.

DISCUSSION

Our results show that tetanic tension of fibres in low $[Na^+]_o$ solutions drops during the tetanus, and that the tension drop is almost certainly the result of failure of activation of the central myofibrils. The conclusion regarding failure of activation is based on two points: (1) fibres tetanically stimulated in normal Ringer show neither a tension drop under isometric conditions, nor wavy myofibrils when they were allowed to contract isotonically; (2) fibres shortening isotonically in low $[Na^+]_o$ solutions develop wavy central myofibrils, which we take as a sign that the fibrils are not activated, at about the same time that tension begins to drop under isometric conditions.

Rüdel & Taylor (1969) and Taylor & Rüdel (1970) have reported that waves develop in tetanically stimulated fibres in normal Ringer when sarcomere length is about 1·6 μm or less. There is only a faint suggestion of these waves in our experiments in normal Ringer, even though sarcomere length falls to less than 1·5 μm, as evidenced by the absence of visible striation spacings. The very clear waves that we see in low $[Na^+]_o$ must, in any case, be quite different in origin from those observed by Rüdel & Taylor, since the number of wavy myofibrils continues to increase even after the end of shortening and they are already visible when the apparent sarcomere length is approximately 1·9 μm. This is close to the sarcomere length of 2 μm which Brown, Gonzalez-Serratos & Huxley (1970) reported as the upper limit for the beginning of wavy myofibrils formation.

If it is assumed that the T-system membrane is not able to produce regenerative action potentials and that its depolarization is purely passive, then the phenomena described in this paper could be explained by a change in the size and shape of action potential: a small action potential of short duration might not sufficiently depolarize the T-system in the centre of the fibre. It was shown in the results section, however, that action potential size and duration are not sufficiently altered by low $[Na^+]_o$ to explain the failure of activation. At the moment when the signs of failure of activation of myofibrils appear, the decreased size of the surface action potential is still greater than the minimum depolarization necessary to activate the central myofibrils in low $[Na^+]_o$ solutions (Costantin, 1970).

If instead, it is assumed that the tubular membrane is able to undergo a regenerative action potential similar to the one of the surface membrane, then our results could be explained in terms of a Na^+ depletion process occurring within the tubular lumen. Depletion favoured by the high surface to volume ratio of the T-system (Peachey, 1965). For depletion to occur assuming electroneutrality, it is necessary that not every Na^+

that crosses the tubular membrane during an action potential be replaced by another Na ion entering the tubular lumen from the external medium.

Although there are several possibilities of ion and particle movement which would involve osmolar equilibrium, swelling and shrinkage, we have chosen a simple model which can reasonably account for the experimental results. In this model the tubules, the external medium, and the myoplasm are considered as three different compartments. Depletion can be explained as follows: each action potential is accompanied by a movement of Na^+ into the myoplasm. In order to preserve electroneutrality cations (Na and Tris) enter the tubular compartment and anions (Cl) move out of it. In consequence, differences in ionic concentrations will be created between the external medium and the tubule that will tend to be compensated by diffusion of the ions down their concentration gradients. If the rate of Na^+ loss exceeds the diffusion rate, the net effect will be a decrease in Na^+ concentration in the tubular compartment. A mathematical treatment of this model is given in the Appendix. In Text-fig. 6 the change in tubular Na^+ concentration is shown as a function of time for different frequencies of stimulation and different external Na^+ concentrations. When the half-times of the decay of tubular Na^+ concentration predicted by the model and the half times of the decrease in tension as given in the results section are compared, it can be seen that at high frequencies of stimulation there is a good agreement, although it deviates at lower frequencies. At present we do not have a simple explanation for this disagreement, but the simplifications introduced in the model could account for it. Nevertheless, the qualitative predictions of the model are correct in that the lower the frequency of stimulation, the larger the half-time of tension decay or the time for the first inactivated myofibrils to appear. In the model, lowering $[Na^+]_o$ slightly decreases the half-time of decay. This is in fair agreement with the experimental results considering the inaccuracy of estimation of the experimental half-times of tension decay. The model also predicts that in normal Ringer solution the tubular Na^+ concentration is decreased by high frequency stimulation. At 60 shocks/sec the tubular Na^+ concentration falls to a steady value of 64·9 mM. At this concentration the size of the hypothetical tubular action potential would not be very much reduced as compared with the action potential of the surface membrane (Nastuk & Hodgkin, 1950).

It is worth mentioning here that if this model is solved for the period after stimulation, the recovery should be slower than is observed experimentally (Text-figs. 1, 2 and 3). This is probably because we have lumped the entire T-tubular system into one compartment. Depletion in a real fibre would begin in the centre of the fibre and spread toward the periphery while recovery would occur in the opposite direction. Since the area

associated with a given fraction of the radius is smaller near the centre compared with the periphery and tension measured is proportional to the mechanically active fractional area, one would expect that the inward recruitment of myofibrils during recovery would be faster than the outward spreading failure of myofibrils during depletion.

In conclusion, we feel that the experiments reported on here support the idea that the inward spread of activation of the contractile mechanism in twitch muscle fibres may well be due to a Na^+-dependent action potential along the walls of the T-tubules.

APPENDIX

The tubular system is considered as a single compartment separated from the myoplasm by the tubular membrane and connected to the external medium through a diffusion barrier. The potential difference across this barrier is probably small and has been neglected. It is assumed that the tubular membrane is excitable as is the surface membrane, but does not have appreciable delayed rectifier. The maximum Na^+ conductance in the tubular membrane is supposed to be the same as in the surface membrane.

The relationship between net Na^+ influx per impulse ϕ_{Na} and tubular Na^+ concentration c_{Na}, was obtained from computations with the Hodgkin & Huxley equations (1952) (although $\bar{g}_{Na}$ was not corrected for changes of c_{Na}) and scaled appropriately for muscle fibres according to the sodium fluxes measurements by Hodgkin & Horowicz (1959). For simplicity, the function was approximated by a logarithmic expression of the type

$$\phi_{Na} = (1{\cdot}33 \ln c_{Na} - 3{\cdot}52)\, 10^{-12} \frac{\text{mole}}{\text{impulse cm}^2} \quad \text{for } c_{Na} \geqslant 13{\cdot}43 \text{ m-mole/l.}$$

and

$$\phi_{Na} = 0 \quad \text{for} \quad c_{Na} < 13{\cdot}43 \text{ m-mole/l.} \tag{1}$$

It has been assumed that the tubular surface is four times larger than the fibre surface. Thus, at $c_{Na} = 120$ m-mole/l. eqn. (1) will give a flux of 3×10^{-12} m-mole per square centimetre of tubular membrane per impulse which is one fifth of the net flux per impulse per square centimetre of muscle membrane (Hodgkin & Horowicz, 1959).

The rate of loss of Na will be given by

$$f \frac{a}{v} \phi_{Na} (1 - T_{Na}) \tag{2}$$

where f is the frequency of stimulation, a/v is the surface to volume ratio of the tubular compartment and T_{Na} is the transference number for Na^+ at the mouth of the tubule. Taking in account diffusion by concentration gradients, the rate of change of c_{Na} in the tubule is given by the equation

$$\frac{dc_{Na}}{dt} = \alpha_{Na}(c^0_{Na} - c_{Na}) - f \frac{a}{v} \phi_{Na}(1 - T_{Na}), \tag{3}$$

where α_{Na} is the rate constant for Na^+ diffusion between the tubular compartment and the external medium and c^0_{Na} is the Na^+ concentration in the external medium. Analogous expressions can be written for the chloride ion concentration, c_{Cl}, and Tris ion concentration, c_T, in the tubular compartment. The transference numbers are given by the following expressions

$$T_{Na} = \frac{c^0_{Na} u_{Na}}{c^0_{Na} u_{Na} + c^0_T u_T + c_{Cl} u_{Cl}},$$

$$T_{Cl} = \frac{c_{Cl} u_{Cl}}{c^0_{Na} u_{Na} + c^0_T u_T + c_{Cl} u_{Cl}},$$

$$T_{Na} + T_{Cl} + T_T = 1,$$

where u_{Na}, u_{Cl} and u_T are the mobilities of Na chloride and Tris ions respectively; c^0_T is the external Tris concentration and c_{Cl} is the chloride concentration in the tubular lumen. To maintain electroneutrality in the tubular compartment it is required that

$$c_{Cl} = c_T + c_{Na}$$

where c_T is the Tris ion concentration in the tubular lumen.

These equations were solved by a predictor–corrector numerical procedure using the following values for the constants:

$$a/v = 10^6 \text{ cm}^{-1} \text{ (Peachey, 1965)},$$

$$u_{Na} = 5{\cdot}19 \times 10^{-4} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1},$$

$$u_{Cl} = 7{\cdot}91 \times 10^{-4} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1},$$

$$u_T = 2 \times 10^{-4} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1}.$$

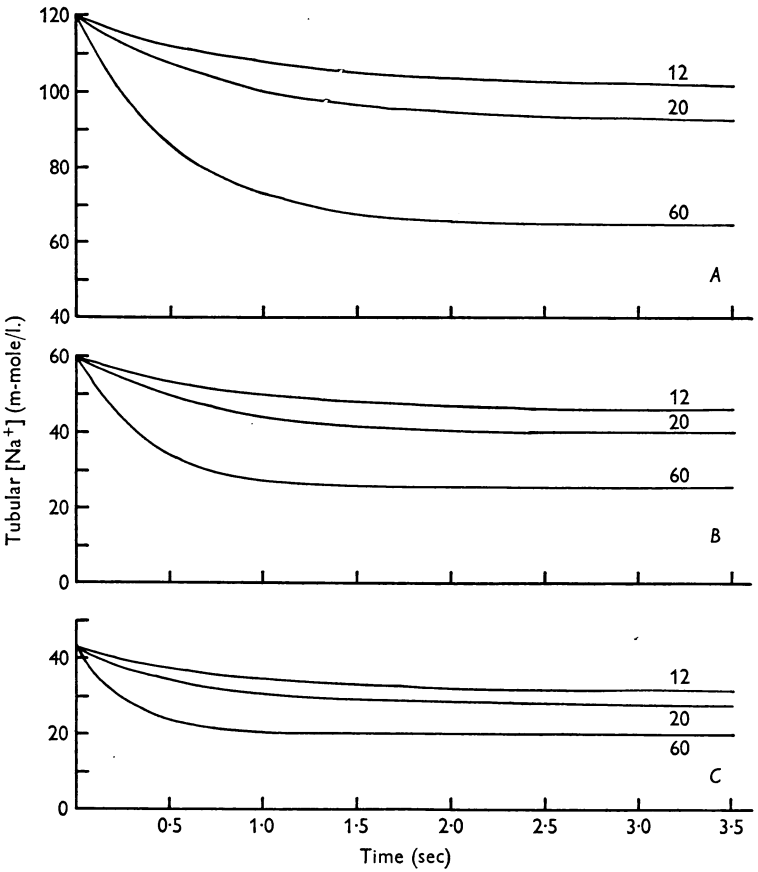
The Tris ion mobility, u_T, was assumed to be half the mobility of hydroxymethyl ammonium ion whose limiting equivalent conductivity is given by Robinson & Stokes (1959).

The values of α_{Na}, α_{Cl}, and α_T were assumed to be all equal to satisfy electroneutrality in diffusion and the value was assumed to be equal to 1 sec^{-1} from the values published for potassium by Hodgkin & Horowicz (1959).

The results are plotted in Text-fig. 6 for a variety of c^0_{Na}, c^0_T and c^0_{Cl} and for different stimulation frequencies.

It is important to note that c_{Na} is the average tubular $[Na^+]$. In reality it would be expected that during repetitive stimulation c_{Na} near the mouth of the tubules be higher than at the centre of the fibre.

R. H. Adrian (personal communication) using a distributed model for the tubular network, has estimated that the maximum Na^+ conductance in the tubular wall would be smaller than the one of the surface membrane; this would decrease the Na^+ depletion during repetitive stimulation. Nevertheless an a/v value moderately larger than 10^6 cm^{-1} would tend to compensate such a difference in sodium conductance. This does not seem unreasonable in view of the complex geometry of the T-system



Text-fig. 6. Time course of the changes of Na^+ concentration in the interior of the tubules at different frequencies of stimulation and at different $[Na^+]_o$ as computed from the model. The frequencies of stimulation in shocks/sec are indicated on each curve. The sodium concentrations are as follows: *A* 120 mM, *B* 60 mM and *C* 43 mM.

(see, for example, Eisenberg & Eisenberg, 1968). On the other hand, it has been reported (Rojas & Canessa-Fischer, 1968) that in squid axons the net entry of Na^+ per impulse is independent of the Na^+ equilibrium potential as judged by changes in internal $[Na^+]$. If a similar independency were present in the T-tubules membrane, the tubular Na^+ depletion would be greater than the ones shown in Text-fig. 6.

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Note added in proof. Since this work was submitted for publication, a model which takes into account isosmolarity, electroneutrality and changes in tubular volume has been solved. The results obtained are similar to the ones presented above, and the recovery after stimulation is faster due to the tubular shrinkage during repetitive stimulation.

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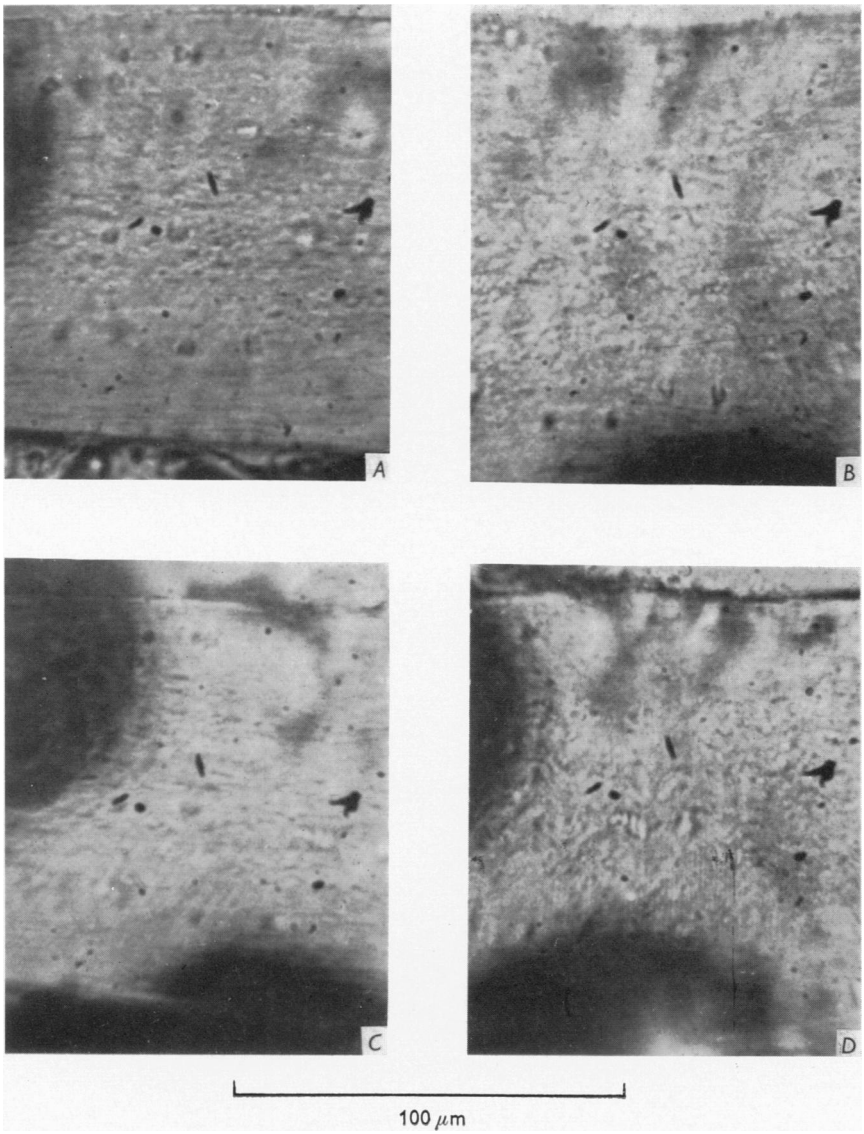
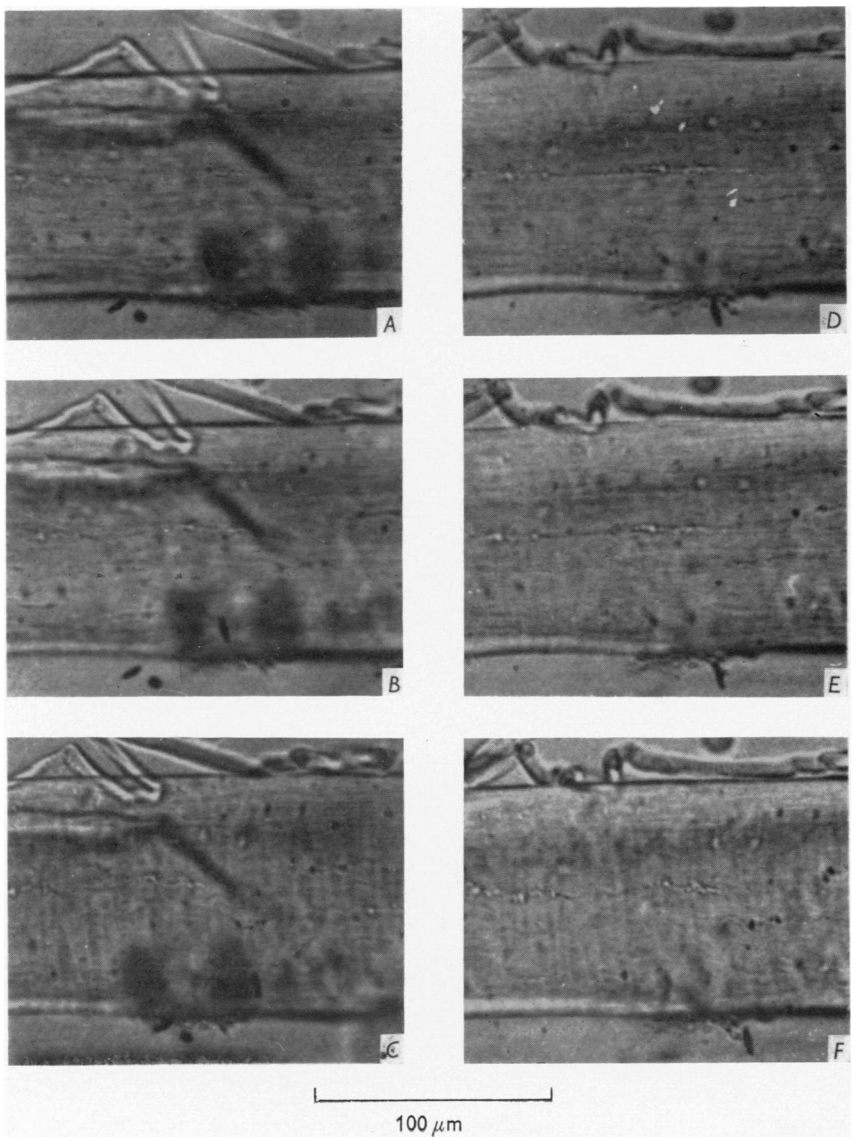

A
B
C
D
100 μm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EXPLANATION OF PLATES

PLATE 1

Sample pictures from a ciné-recording (100 frames/sec) of an isotonic contraction of an isolated muscle fibre during tetanic stimulation (70 shocks/sec). In Figs. *A* and *B* the fibre was in normal Ringer solution and in *C* and *D* in 62·5 mM-[Na^+] solution. The times at which the pictures were taken after the beginning of the tetanus were as follows: *A*, 246 msec; *B*, 965 msec; *C*, 211 msec and *D*, 945 msec. Fibre 28. v. 71. Calibration bar 100 μm. Water immersion objective × 40, N.A. 0·75.

PLATE 2

The development of wavy myofibrils of an isolated muscle fibre stimulated at 50 shocks/sec in two different Na^+ concentrations. In Figs. *A–C* the fibre was in a solution containing 51 mM-[Na^+] and in Figs. *D–F* it was in 62·5 mM-[Na^+]. The times at which the photographs were taken are as follows (in msec): *A*, 170; *B*, 214; *C*, 380; *D*, 260; *E*, 375; *F*, 970. Fibre 21. v. 71. Calibration bar 100 μm. Water immersion object × 25, N.A. 0·40.